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UTILIZATION OF SOME FRUITS POMACE AS NATURAL ANTIOXIDANTS TO SHELF-LIFE OF SUNFLOWER OIL

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ABSTRACT

Apple, orange and pear pomance a by – products of fruits juice industry are rich sources of fiber and polyphenols. Therefore, the chemical compositions, minerals and fiber fractions were determined in the fruits pomaces. The results showed that the apple pomance the highest protein content 9.86%. Also, the orange and pear pomance powder were contained nearly in crude fiber 8.34 and 6.75% respectively. Moreover, apple pomance was the highest total flavonoids and total phenolic compound (4.15 and 9.72 mg/g). Meanwhile, orange is a good source of vitamin C (0.42 mg/g) and pear pomance was the highest content of total carotenoids 765.51 ug/g.

Fractionation of polyphenols content was relatively determined using HPLC to the extracts of apple, orange and pear pomance. Apple pomance extract contained highly amounts of kaempferol, cumarin o-cumaric, rutin and chlorogenic acid. Orange extract contained ferulic, salcylic and o- cumaric acids and pear contained resorcinol, rutin, myricetin, quercetin and kaempferol.

Antioxidants activity were determined from extracts of all fruits pomance and compared with butylated hydroxy anisol (BHA) as a synthetic antioxidant. The results showed that the BHA represented 92.61%, activity followed by apple pomance extract and orange extract which represented 91.2 and 85.38% activity.

Different concentrations (100, 200 and 250 ppm) of all fruits pomance extract compared with BHA at level 250 ppm were added to sunflower oil. The stability of all samples measured the changes in peroxide value during incubation of heating period. The results

indicated that very close effects were observed for the BHA and natural extract from apple and orange at level 250 ppm.

INTRODUCTION

In the recent year, there has been a global trend toward the use of natural phytochemicals present in natural sources, such as vegetable, fruits, herbs, cereals, grains and oil seeds, as antioxidant agents and functional foods (Lee et al., 2002). Natural antioxidant can be used in the food industry and there are many evidences that substances may exert their antioxidant effects within the human body. They effectively prevent premature of lipid oxidation. It is known that lipids autoxidation readily, once isolated from plant materials and used in processed food. As a result, organoleptically and nutritional quality is reduced and even toxic products may be formed. The retardation of autoxidation is therefore a key to high product quality. Because most consumers prefer natural food additives over synthetic ones, natural antioxidants have increasing importance (Kings and Berger, 2001).

Meydani (2001) suggested that vitamin radicals (tocopherol radical) act as per oxidants during the autoxidation of LDL. It was also shown that shortened leg time induced by higher doses of vitamin E was restored when lipid and water soluble antioxidant were added simultaneously. Also, which suggest that vitamin E radicals derived from vitamin E are subsequently reduced by vitamin C to regenerate vitamin E. Thus, the interaction between lipid and water soluble antioxidants provides an important function in maintaining LDL resistant to oxidation.

Food-derived flavonoids such as the flavonols, quericetin, haempferol and myricetin have been reported to reduce cancer risk. Querecetin and kaempferol are typical flavonols and their corresponding flavones flavonos are luteolin and apigenin. The average intake of all flavonoids in the western diet is estimated to be 1g/day. Consumption of vegetables and fruits is found to have protective effects on various forms of cancer in addition to providing vitamin C and β-carotene (Chu et al., 2000).

Knekt et al. (2002) cited about the relation between flavonoids and other dietary phenolic constituents to the protective effect. As they act as antioxidants by virtue of the free radical scavenging properties of their constituent hydroxyl group. The extended conjugation across the flavonoid structure and also the increasing

number of the hydroxyl groups enhance the antioxidant properties, allowing them to act as reducing agents or single oxygen scavenging.

The aim of this investigation was to recycle apple, orange and pear pomaces a by-product of fruits juice industry for new products, which beneficial effects on human health.

MATERIALS AND METHODS

Materials:

Apple (Malus domestice), orange (Citrus sinensis L.) and pear (Pyrus communis L.) fruits pomance were purchased from Aga Company, Daquahleya Governorate Egypt. All fruits pomance were washed several times and dried in an air oven at 60C for 12 hr. The dried fruits pomance were milled in Laboratory Quadramate Mill Junior to give a fine powder. After that the fruits pomance powder were packed in poly ethylene bags and stored in refrigerator at 5C for analysis.

Sunflower oil (free from antioxidant) was obtained from Tanta Oils and Soap Co., Banha Factory, El-Kaliubia Governorate, Egypt. Wheat variety Sakha 69 cultivar was obtained from Field Crop Research Institute. Wheat was milled in Laboratory Quadramate Mill Junior to give wheat flour extraction 72%.Whereas, Butyl hydroxyanisol (BHA) was obtained from Noarden International Company, Holland.

Methods:

Chemical analysis of fruits pomance:

Protein, oil, ash and crude fiber were determined according to the methods of the AOAC (2000). Total carbohydrates were determined by differences. Whereas, ascorbic acid (Vit. C) was determined using 2,6 dichlorphenol –indophenol titration method according to the methods described Ranganna, (1979). Also, the method described by Wettestein (1957) was used for the determination of carotenoids. Total phenols were determined using Folin- Dens reagent according to the method of Valverde et al.,(2002). Whilst, total flavonoids was determined according to AOAC (2000).Total mineral contents of zinc, iron, calcium, cupper and selenium were determined in samples, according to Galvao et al.

(1976) using Perkin Elier Atomic Absorption Spectrophotometer model 80, England.

Extraction of antioxidants:

Extraction and isolation of antioxidants from native materials were carried out according to the procedures of Mabry et al. (1970).

Fractionation of polyphenols by HPLC

High performance liquid chromatography (HPLC) technique (Hewlett Packard series 1100 HP 1100). Column hypersil BDS 5 um C 18. Detector UV 254nm. Flow rate 0.3ml/min. Mobile phase A:(0.5 ml acetic acid/99.5 ml distilled water). B: (0.5 ml acetic acid/99.5 ml acetonitrile). Temperature ambient 25C.(Merfort et al., 1997).

Determination of antioxidant activity

The antioxidative activity of the obtained antioxidant from fruits pomance were determined, comparing with butylated hydroxylanisol (BHA), using the thiocyanate method which described by Tsuda et al. (1993).

Addition of antioxidant to sunflower oil

Sunflower oil was used as a substrate for oxidation studies. Antioxidant extracts prepared from samples of the investigated raw materials and synthetic antioxidant butyl hydroxyanisol (BHA), were added to oil at 100, 200, 250ppm levels to test their antioxidant effectiveness according to Buford (1988). Control sample with no additive was used under the same conditions. Sunflower oil with or without antioxidant was heated in 500ml. glass beaker (97cm^2 surface area) at 180°C ± 5°C for 24hr. (total heating hours). Intermittent heating period was 4hr/day. After heating, the oil samples were taken periodically and stored in glass bottles at -10°C till analysis for peroxide value. The peroxide value was determined according to the method described in AOAC (2000).

RESULTS AND DISCUSSION

Chemical composition of apple, orange and pear pomances:

From the results in Table (1), it could be observed that the apple dry pomance had the highest content of protein 9.86% followed by orange pomance 7.55%. Also, the orange and pear dry powder were contained nearly in crude fiber 8.34 and 6.75%, respectively followed by apple dry powder 5.72%. Moreover, pear dry powder had the

highest total carbohydrates 84.31% followed by apple and pear contained 80.97 and 79.03%, respectively. Total flavonoids, total phenolic, ascorbic acid and total carotonoids were determined in the apple, orange and pear pomances powder. The results showed that the apple pomance powder was the highest in total flavonoids and total phenolic (4.15 and 9.72mg/g) followed orange powder and pear pomance powder. These increases in total flavonoids and total phenolic my be dependent on the fruit coats color. Where as the orange is a good source of vitamin C and the orange pomance powder had the highest content of ascorbic acid 0.42mg/g followed by apple pomance 0.27 mg/g and pear 0.11 mg/g. Mean While, pear pomance powder showed the highest content of total carotonoids 765.51 ug/g followed by apple pomance 465.86 ug/g and orange powder 269.45 ug/g. These results confirmed those obtained by Sudha et al., (2007) who found the apple pomance, a by – product of apple juice industry is a rich source of fiber and polyphenols. Also in view of the antioxidant properly of pomance, it would play on important role in prevention of diseases. Apple pomance procured from fruit juice industry, contained 10.8% moisture, 0.5% ash, 51.1% of dietary fiber and 10.1% protein.

Ascorbic acid (vitamin C) is a water- soluble essential nutrient and perhaps the most important antioxidant in extra cellular fruits. It was found to be the most effective in inhibiting lipid peroxidation initiated by peroxyl radical initiator among several types of antioxidant including ∞ - Tocopherol (Niki et al., 1995).

Carotenes have a positive effect on the immunological system and protect the skin from ultraviolet radiation (Bendich, 1989). Some carotenoids have pro- vitamin A activity which is of particular interest in those population that are vitamin A deficient. Carotenoids are excellent antioxidants and could have many health benefits.

Table (1) Chemical composition of apple, orange and pear pomances (on dry weight bases g/ 100g).

Chemical Compositions	Apple	Orange	Pear
Protein	9.86	7.55	5.72
Oil	1.57	1.52	0.81
Ash	1.88	3.56	2.41
Crude fiber	5.72	8.34	6.75
Total carbohydrate	80.97	79.03	84.31
Total flavonoids (mg/g)	4.15	2.72	1.27
Ascorbic acid (mg/g)	0.27	0.42	0.11
Total phenolic (mg/g)	9.72	7.15	4.38
Total carotenoids (ug/g)	465.86	269.45	765.51
Zinc (mg/ 100 g)	7.25	5.98	3.08
Iron (mg/ 100 g)	580.8	137.5	420.8
Calcium (mg/ 100 g)	763.5	192.75	82.50

The minerals content were determined in apple, orange and pear pomance and the results are recorded in the same Table, it could be observed the apple pomance had the highest content of zinic (7.25ppm) followed by orange and pear pomance (5.98 and 3.08 ppm respectively. The highest iron content was observed for apple pomance (580.8 ppm) followed by pear and orange (420.8 and 137.5 ppm, respectively). However, calcium contained the highest values of apple pomance (763.5 and 244.1 ppm, respectively) followed by orange and pear pomance.

Minerals and trace elements are essential nutrients for good preventive nutrition. Calcium is required for formation and maintenance of skeleton, muscle, heart, nervous and blood clotting. Also magnisum is implicated in regulation of nervous system and prevents spasmophila. Whereas, iron is incorporated in the

hemoglobin molecule and plays a role in the transport of oxygen. Zinc and copper act as constituents of a number of enzymes (Lopes et al., 2003).

Apple, orange and pear extract using HPLC:

Data in Table (2) showed that the both of polyphenols and flavonoids content were relatively high in ethanol extract (80%). Apple pomance extracts had contained relatively high amounts of kaempferol (10.99%), cumarin (34.17%), 0- cumaric acid (9.05%), rutin (1.743%) and chlorogenic acid (9.33%) results in the same table indicated that orange extract contained high amounts of ferulic, salicylic and o – cumaric acids (67.86, 8.04 and 8.89%, respectively). whereas, pear pomance extract had high amounts of rsoricnol (8.54%) , retin (8.06%), myricetin (29.69%), quercetin (8.15%) and kaepferal (9.72%).

Under these experimental condition, some of compounds were absent such as pyrogallic, gallic and hydroquinone acid in apple extract whereas, pyrogallic, gallic acid and cumarin were not found in orange extract and also, gallic and protocatechuic acids not show up in pear pomance extract. These results are parallel with data obtained by Ma et al., (2008) they found seven phenolic compounds of two families including cinnamic acids (caffeic, p- coumaric, ferulic, sinapic acids) and benzoic acids (protocatechuic, p- hydroxybenzoic, vanillic acids) from citrus (citrus unshiu Marc) peels were evaluated by Mltrasound – assisted extraction (UAE).

Antioxidant activity of fruits pomance extracts:

Antioxidants activity were determined for extracts obtained from apple, orange and pear pomances and compared with butylated hyderoxyanisol (BHA) as synthetic antioxidant. The results are recorded in Table (3). From the results it could be noticed that activity in the linoleic acid system of control (no additive) represents 100% lipid peroxidation with absorbance value of 0.65 at 500 n.m., this mean that antioxidant activity equal 0.00% where as, the synthetic antioxidant, butylated hydroxyanisol (BHA) showed 92.6% activity followed by apple pomance extract which showed 91.2% activity. Moreover, orange extracts recorded 85.38% activity, while pear pomance extract showed 82.77% activity. These results agreed with those reported by Tsao et al., (2005) they found the antioxidant capacity of apple pomance is related to its phenolic profile.

Procyanidins have long been recognized as the major contributors to antioxidant activity of apples and derivatives (Oszmianski et al., 2008), which capacity depends on their polymerization degree and substitutions (lotito et al., 2000). Also, the antioxidants activity of hydroxycinnamic and benzoic acids and flavonols has been ascertained (Tsao et al., 2005).

The effect of fruits pomance extract on oxidative stability of flower oil:

Different concentrations (100, 200 and 250 ppm) of fruits pomance extract in addition to butylated hydroxyanisol (BHA) at level of 250 ppm were added to sunflower oil. The stability of all samples were determined by measuring the changes in peroxide value during incubation of heating period and the obtained results are presented in Table (4). The results indicated that when sunflower oil samples were treated with apple and orange extracts as natural antioxidant and BHA as synthetic antioxidant, the peroxide value were lowered than without and other addition. Very close effects were observed for the addition of BHA and natural antioxidant at 250 ppm. this means that apple and orange extract contained antioxidant which lead to retard lipid peroxidation during continuous heating. However, the peroxide values of sunflower oil samples were 7.44, 7.85 and 11.78 milliequivalant / kg with addition of 250 ppm of apple, orange and pear extracts compared with 7.31 milliequivalant / kg for BHA at the same concentration (250 ppm).

Oxidative is one of the most important processes of food deterioration because it may effected food safety, color, flavor and texture Antioxidants may protect food quality by preventing oxidative deterioration lipid (Jienez and Garcia – Caromona, 1999).

The intake of plant antioxidants has been shown to be inversely related to the risk of cardiovascular disease. Several flavonoids have been reported to quench active oxygen species and inhibit in vitro oxidation of low density lipoproteins (LDL) and therefore, reduce thermo biotic tendency. Flavonoids may inhibit thereby modulate metabolism of arachidonic acid and attenuate inflammation. Many plant antioxidants (flavonoids have also been showed to be anti – carcinogenic in several animal models (Miean and Mohamed, 2001).

Table (2) Fractionation of polyphenols content from apple, orange and pear extracts using HPLCl. (%)

Compounds	RT	Apple	Orange	Pear
Pyrogallic	5.297	_	4.657	_
Hydroquinone	5.918	_	4.989	0.504
Gallic	7.310	_	_	_
Resrocenol	9.565	1.441	8.542	2.359
Protocatechuic	11.142	0.214	_	2.436
OH- Benzoic	14.426	2.297	1.933	0.335
Chlorogenic	16.380	9.327	1.759	0.505
Catechin	16.814	1.682	1.985	0.644
Phenol	18.399	1.128	1.609	0.701
Vanillin	19.156	1.010	1.470	0.477
P-coumaric	21.025	1.590	2.342	1.234
Ferulic	22.465	1.195	5.002	67.863
Salicylic	22.975	1.653	2.042	8.040
Rutin	24.006	17.43	8.064	0.835
O-coumaric	25.243	9.048	5.551	8.890
Coumaric	25.718	34.174	_	_
Myricetin	28.030	2.922	29.62	0.387
Apigenin	29.368	1.233	1.258	2.115
Cinnamic	31.406	0.8552	1.282	0.545
Quercetin	32.213	0.814	8.145	1.005
Kaempferol	36.286	10.99	9.718	1.126

Rt, Retention time.

Table (3) Antioxidant activity of apple, orange and pear pomance extracts and compared with butylated hydroxy anisol (BHA).

Antioxidants	Absorbance at 500 n.m	Lipid peroxidation	Activity %
No additive	0.65	100	0.00
BHA	0.048	7.38	92.6
Apple	0.057	8.77	91.2
Orange	0.095	14.62	85.38
Pear	0.112	17.23	82.77

Table (4) Oxidative stability of sunflower oil treated with different pomance extracts compared with BHA

Samples	Time after 4 hr / Day						
	0	4 hr	8 hr	12 hr	16 hr	20 hr	24 hr
Sunflower oil	**0.98**	**4.01**	**6.55**	**9.92**	**14.59**	**19.00**	**24.28**
BHA							
250 ppm	**0.98**	**2.13**	**3.76**	**4.95**	**4.95**	**6.67**	**7.31**
Apple extract							
100 ppm	**0.98**	**3.82**	**4.45**	**5.85**	**5.85**	**7.42**	**8.74**
200ppm	**0.98**	**3.25**	**3.63**	**4.15**	**4.15**	**5.93**	**7.12**
250 oon	**0.98**	**2.95**	**3.11**	**3.92**	**3.92**	**5.46**	**7.44**
Orange extract							
100 ppm	**0.98**	**4.55**	**4.95**	**7.24**	**7.24**	**7.82**	**9.71**
200 ppm	**0.98**	**3.84**	**3.88**	**4.98**	**4.98**	**6.52**	**8.12**
250 ppm	**0.98**	**3.40**	**3.73**	**4.97**	**4.97**	**6.31**	**7.85**
Pear extract							
100 ppm	**0.98**	**5.73**	**7.41**	**11.41**	**11.41**	**13.15**	**15.34**
200 ppm	**0.98**	**4.78**	**6.98**	**9.52**	**9.52**	**11.12**	**13.56**
250 ppm	**0.98**	**4.12**	**6.12**	**8.73**	**8.73**	**9.82**	**11.78**

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الاستفادة من بعض مخلفات الفاكهة كمضادات أكسدة طبيعية لإطالة فترة ثبات زيت عباد الشمس

عبد المنعم صالح إبراهيم صالح الشاذلي

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تعتبر مخلفات الفاكهة (تفاح-برتقال-كمثرى) هى منتج ثانوى من تصنيع العصائر كما ان هذه المخلفات غنية بالالياف والمركبات البولى فينوليه .لذلك تم تحليل التركيب الكيماوى والمعادن وتفريد الالياف لهذه المخلفات . أوضحت النتائج ان مخلف التفاح غنى بالبروتين 9,86 %كما ان مخلف البرتقال والكمثرى يحتوي على نسبه عاليه من الالياف 8,34 و 6,75 % على التوالى . بالاضافه الى ان التفاح يكون غنى بالمركبات الفلافونيديه والفينولات الكليه 4,15 و 9,72 مليجرام/جرام على التوالى أما مخلف البرتقال فهو غنى بفيتامين C 0,42 مليجرام/جرام والكمثرى يكون مرتفع فى الكاوتينات الكليه 765,51 ميكروجرام/جرام.

أوضحت النتائج ان تفريد البولى فينول تم بواسطه جهاز HPLC وجد أن مخلف التفاح يحتوى على الكامفيرول – الكيومارين –الريوتين وحامض الكلوروجنيك اما مخلف البرتقال فوجد ان يحتوى على الفيروليك – السالسليك والكيوماريك وقد وجد ان مخلف الكمثرى غنى بالريوتين-الميرسينين-الكورسين والكامفيرول .

تم تقدير نشاط مضادات الاكسده الطبيعيه ومقارنتها بالمضادات الاكسده الصناعيه (BHA) واوضحت النتائج ان BHA اعطى اعلى نشاط 92,61 % يليه التفاح 91,2 % ثم البرتقال 85,38%.

اوضحت النتائج لاضافه مستخلص هذه المخلفات على نسب 100-200-250 جزى فى المليون لزيت عباد الشمس لاختبار ثبات زيت عباد الشمس مع مقارنتها بأضافه 250 جزء فى المليون لمركب BHA وتم تقدير رقم البيروكسيد اثناء فتره التسخين فوجد انه لا توجد اختلافات واضحه بين BHA ومستخلص مخلف التفاح غينه والبرتقال على 250 جزء فى المليون .